

# Effects of Probiotic Consumption on Absence Seizures

## Probiyotik Tüketiminin Absans Nöbetler Üzerine Etkisi

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### Summary

**Objectives:** Probiotics are microorganisms of intestinal microflora that are beneficial for human health. Childhood absence epilepsy has 2 validated rat models: Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo from Rijswijk (WAG/Rij). To date, there have been no clinical or experimental studies of the effects of probiotics on absence epilepsy. The present study was an investigation of the effects of probiotics on absence seizures in the GAERS rat model.

**Methods:** GAERS were used to examine the effects of probiotics. Nine male GAERS were assigned to 1 of 2 groups (probiotic or control). The animals had free access to food and water. Commercially available probiotic product was provided in drinking water to probiotic group for 1 month. Surface electrodes were then implanted for electroencephalogram (EEG) recordings. Two aspects of EEG recordings were compared: cumulative duration and cumulative number of absence seizures.

**Results:** Analysis of spike-and-wave discharges between the 2 groups showed no significant difference in either cumulative duration or number ( $p>0.05$ ). Additionally, it was observed that probiotic group consumed more water than control group ( $p<0.05$ ).

**Conclusion:** Results indicated that probiotic consumption had no effect on duration or number of spike-and-wave discharges of GAERS after 1-month feeding period. This is the first investigation in the literature addressing interactions between probiotics and absence epilepsy, and further research is needed.

Keywords: Childhood absence epilepsy; Genetic Absence Epilepsy Rats from Strasbourg; probiotics; rat.

### Özet

**Amaç:** Probiyotikler, insan sağlığına yararlı ve bağırsak mikroflorasında yaşayan canlı organizmalardır. Çocukluk çağı absans epilepsisinin iki adet iyi tanımlanmış modeli mevcuttur: Genetic absence epilepsy rats from strasbourg (GAERS: Strasbourg'dan genetik absans epilepsi sıçanları) ve Wistar Albino Glaxo from Rijswijk (WAG/Rij: Rijswijk'den Wistar Albino Glaxo). Bugüne kadar, probiyotiklerin absans epilepsisi üzerine etkileriyle ilgili klinik veya deneysel bir çalışma yapılmamıştır. Bu çalışmada, absans epilepsisinin sıçan modeli olan GAERS üzerinde probiyotiklerin absans nöbetleri üzerine etkileri incelendi.

**Gereç ve Yöntem:** Absans epilepsi modeli GAERS sıçanlar, probiyotiklerin etkilerini gözlemlemek için kullanıldı. Dokuz erkek sıçan iki gruba (Probiyotik ve Deney) ayrıldı. Su ve besine limitsiz ulaşimleri sağlandı. Deney grubu bir ay süreyle ticari olarak satılan probiyotik ürününü içeren su ile beslendi. Sonrasında elektroensefalografi (EEG) kaydı için stereotaktik cerrahi ile kortikal kayıt elektrotları implante edildi. Elektroensefalografi kayıtları nöbetlerin kümülatif süresi ve sayısı açısından karşılaştırıldı.

**Bulgular:** Probiyotik ve standart beslenen grupların diken-ve-dalgı deşarjlarının analizi, iki grup arasında kümülatif süre ve sayı açısından bir fark göstermedi ( $p>0.05$ ). Diğer taraftan su tüketimi probiyotik ile beslenen grupta daha fazla oldu ( $p<0.05$ ).

**Sonuç:** Çalışmada, bir aylık probiyotik tüketiminin GAERS modelinde gözlenen diken-ve-dalgı deşarjları üzerine etkisi olmadığı gösterildi. Bu çalışma, absans epilepsisi ve probiyotikler arasındaki olası ilişkiyi göstermeyi hedefleyen ilk araştırma olup daha ileri çalışmalara-yaklaşımlara ihtiyaç bulunduğunu işaret etmektedir.

Anahtar sözcükler: Çocukluk çağı absans epilepsisi; genetic absence epilepsy rats from strasbourg; probiyotik; sıçan.

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## Introduction

Probiotics are defined as viable microorganisms which, when administered in adequate amounts, confer a health benefit on the host.<sup>[1,2]</sup> Many microorganisms are classified as probiotics, for example, many strains of *Lactobacillus* and *Bifidobacterium*, certain *Enterococci*, and *Escherichia coli* strains.<sup>[3]</sup> *Lactobacillus* and *Bifidobacterium* are the strains that have been most extensively investigated.<sup>[3]</sup> Probiotics are found in many commercially available functional foods, drugs, and dietary supplements, used naturally or intentionally.<sup>[1]</sup>

The Classification of Epilepsies and Epileptic Syndromes written by the International League Against Epilepsy defines childhood absence epilepsy as idiopathic generalized epilepsy characterized by typical absence episodes. During these episodes, the electroencephalographic (EEG) pattern is bilateral, synchronous, and shows symmetric spike-and-wave discharges (SWDs) at 3 cycles per second.<sup>[4]</sup> There are 2 well-described rat models of absence seizures. They are the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo from Rijswijk (WAG/Rij) rat models.<sup>[5]</sup> Both models reflect absence epilepsy pharmacologically, morphologically, and electrophysiologically.

Experimental studies on absence seizures have investigated the underlying electrophysiological cause(s) of SWDs.<sup>[6]</sup> Early studies suggested abnormal cortical and/or thalamic activity of neuron groups producing gamma-aminobutyric acid (GABA) may underlie absence seizures in GAERS. In 1 of these studies, Spreafico et al. demonstrated indirect evidence of reduced GABA-A receptor function in the cerebral cortex of GAERS.<sup>[7]</sup> Later investigations also revealed alterations of GABA-A receptor activity in GAERS.<sup>[8]</sup> Increased number of GABA-B receptors was also observed in the cortex of lethargic (lh/lh) mice.<sup>[9-11]</sup> Princivalle et al. showed up-regulation of GABA-B receptor subunits in the corticothalamic circuit in GAERS.<sup>[12]</sup>

Since there is growing scientific evidence about possible complementary use of probiotics in gastrointestinal diseases, recent studies done to investigate effects of probiotics on neurological and psychiatric disorders are increasing.<sup>[13-15]</sup> Research on effects of probiotics has demonstrated that probiotic consumption may result in alterations in the composition of neurotransmitters and receptors in different brain regions. Ingestion of *Lactobacillus rhamnosus* (JB-1)

affected GABA mRNA expression in a mouse in the vagus nerve.<sup>[16,17]</sup> These studies showed reduced GABA-A $\alpha$ 2 mRNA expression in the prefrontal cortex and amygdala, but increased GABA-A $\alpha$ 2 in the hippocampus. Other findings of these studies indicated that GABA-B1b mRNA was increased in the cingulate and prelimbic cortices and was reduced in the hippocampus, amygdala, and locus coeruleus.

The alterations in GABA receptor expressions in different brain regions after consumption of different probiotic strains may influence occurrence of absence seizures. This possible effect can be quantified by measuring cumulative duration and number of SWDs of GAERS.

To date, there are no clinical or experimental studies of positive or negative effects of probiotics on absence epilepsy. The aim of the present study was to investigate effects of probiotics on absence seizures in a well-described rat model of absence seizure, GAERS. SWDs were recorded and compared according to cumulative duration and cumulative number.

## Materials and Methods

In order to observe effects of probiotic consumption on SWDs, which define onsets and offsets of absence seizures, SWDs of 2 groups of GAERS, probiotic-fed and controls, were compared. Nine male GAERS were used to measure SWDs on EEG recordings corresponding to absence seizures in the experiment. Age of the animals ranged between 5 and 12 months, weight of the animals was between 200 and 250 g each. Animals were maintained under standard laboratory conditions in temperature-controlled room (20 $\pm$ 3 $^{\circ}$ C) with 12-hour light/dark cycle. The experiment protocol was approved by Marmara University Ethics Committee for Experimental Animals (63.2014.mar, 02.10.2014) and all experiments were performed in accordance with international principles of care and use of experimental animals. Animals were separated into 2 groups. The first group was the Probiotic-fed group (n=4) and the second group was the Control-fed group (n=5). Both groups had unlimited access to food and water. Sachet containing 2 g of various probiotic strains, vitamins, and fiber (NBL Probiotic Gold; Cell Biotech Co. Ltd., Gyeonggi-do, South Korea) was dissolved in 500-mL bottle of drinking water provided to just probiotic group. The ingredients of probiotic sachet are provided in Table 1. Bottles were replaced twice a week. Liquid consumption of each group was recorded.

**Table 1.** Ingredients of one probiotic sachet

Ingredients	Amount per sachet
Fiber (fructooligosaccharide, polydextrose)	962.8 mg
Probiotic strains ( <i>Enterococcus faecium</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus rhamnosus</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium bifidum</i> )	2.5x10 <sup>9</sup> cfu
Vitamin C (L-ascorbic acid)	75 mg
Vitamin E	7.5 mg
Vitamin B6	1.98 mg
Vitamin A	0.6 mg
Vitamin B2	1.6 mg
Vitamin B1	1.4 mg

### Surgery

Before surgery, rats were anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Animals were placed in a stereotaxic instrument (Model 51600; Stoelting Co., Wood Dale, IL, USA) and the scalp was longitudinally incised for the implantation of stainless steel screws for epidural EEG recording. Stainless steel screws with soldered insulated wires were bilaterally placed over the frontal and parietal lobes. Microconnector was soldered to tips of cortical electrodes and fixed to the skull with dental acrylic. Following surgery, the animals were housed singly for recovery period of 1 week before recording cortical activities.

### EEG recordings

Electrical activity of the cortex was recorded in bipolar configuration between frontal and parietal tips of the electrodes. Electrical activity was amplified using ML136 Animal Bio Amp (ADInstruments, Dunedin, New Zealand), filtered between 0.3 and 120Hz, digitized at 1000 samples/second, and recorded with PowerLab 8S system running Chart 5 (ADInstruments, Dunedin, New Zealand). SWDs were detected visually on EEG. Criterion for SWD was high-amplitude asymmetric synchronized rhythmic discharge lasting at least 1 second.<sup>[18]</sup>

EEG recordings of every animal were monitored for 3 consecutive days over 3-hour period between 9 am and noon. Each recording was divided into 20-minute periods. Cumulative duration and number of SWDs were calculated for each period and for each individual animal. Subsequently,

mean cumulative duration and number of SWDs for the 2 groups were calculated.

### Statistical analysis

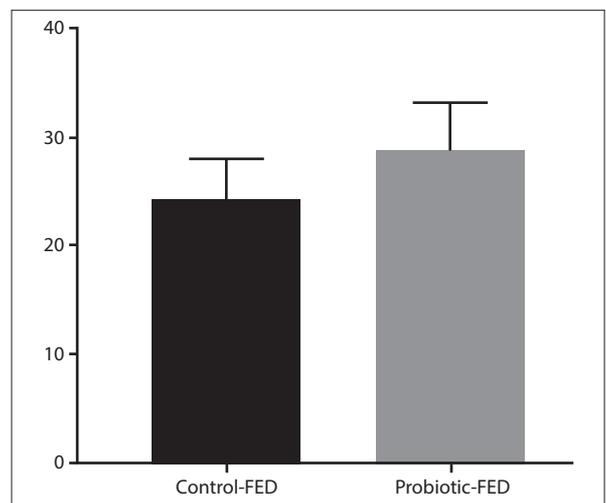
Cumulative duration and number of SWDs were statistically evaluated using repeated measures analysis of variance followed by post hoc Bonferroni tests. Comparison of water consumption was evaluated for each animal per day using Student's t-test. All results were expressed as mean±standard error of the mean. Level of significance was  $p < 0.05$ .

### Results

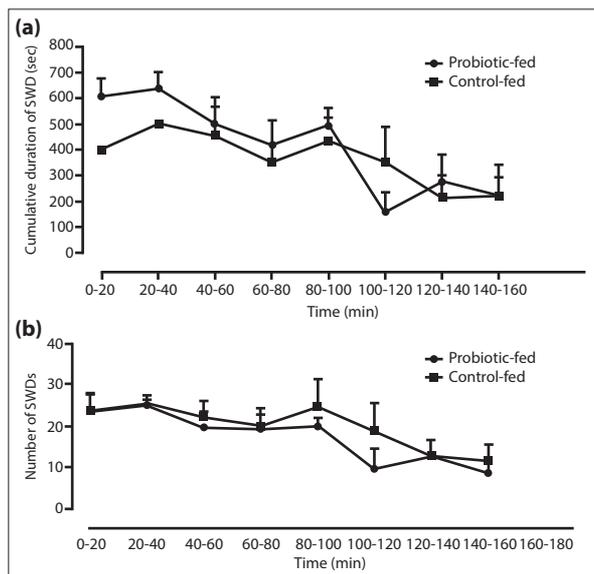
Figure 1 illustrates mean water consumption of animals per day. Statistical analysis demonstrated that water consumption of probiotic group was higher than water consumption of control group ( $t=2.48$ ;  $p=0.01$ ).

For each 20-minute period, cumulative duration and cumulative number of SWDs were calculated and results for 2 groups were compared. No statistically significant difference was found between groups in either comparison ( $p=0.18$  for cumulative duration;  $p=0.66$  for number of SWDs). Cumulative duration and number of SWDs of Probiotic-fed and Control-fed groups in 20-minute periods are provided in Figure 2.

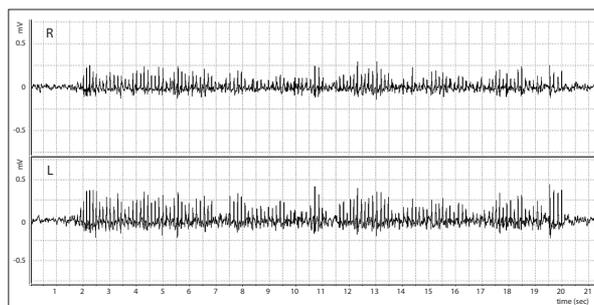
Figure 3 is sample of SWD recorded in one of the control



**Fig. 1.** Mean fluid consumption per animal per day (mL/24h) in Control-fed group and Probiotic-fed group, which was given probiotic mixture in water (Control-fed: 24.31±3.7 mL, Probiotic-fed, 28.67±4.5 mL;  $t=2.48$ ;  $p=0.01$ ).



**Fig. 2.** (a) Mean cumulative duration of spike-and-wave discharges (SWDs) of 2 groups for every 20 minutes of 3 hours of recording. (b) Mean number of SWDs of 2 groups for every 20 minutes of 3 hours of recording.



**Fig. 3.** Bilateral electroencephalogram (EEG) trace of example of spike-and-wave discharge (SWD) from one of the control animals. Onset and offset of this SWD are 2<sup>nd</sup> and 20<sup>th</sup> seconds of the trace, respectively. Top EEG line shows right (R) cortex, bottom EEG line shows left (L) cortex.

animals. In this example, SWD starts and ends at 2<sup>nd</sup> and 20<sup>th</sup> seconds, respectively. Thus, duration of this particular SWD was 18 seconds.

## Discussion

This study was an investigation of effects of probiotics on absence seizures in GAERS, a well-described rat model of absence seizure. With increased interest in probiotics, number of investigations using animal models and humans is increasing in effort to explore possible advantageous effects of different strains of probiotics on several acute and chron-

ic diseases, including diarrhea, irritable bowel syndrome, inflammatory bowel disease, non-alcoholic fatty liver disease, and colorectal cancer.<sup>[19-23]</sup> For central nervous system diseases, studies have investigated efficacy of probiotics on hepatic encephalopathy, depression, chronic fatigue syndrome, and autism spectrum disorder.<sup>[13-15]</sup> However, search of the literature revealed no study similar to ours, which examined effects of probiotics on absence seizures. Therefore, to our knowledge this study will provide new scientific information in this field.

Several studies have demonstrated that feeding probiotics to mice and rats alters the composition of neurotransmitters and their receptors in different brain regions. *Bifidobacterium longum* subsp. *infantis* str. 35624 reduced dopamine and 5HT metabolites in the frontal cortex of rats, but without any discernible change in rat behavior.<sup>[24]</sup> *Bifidobacterium longum* str. NCC3001 repressed anxiety-like behavior and normalized brain-derived neurotrophic factor expression in the hippocampus of mice with mild to moderate colitis.<sup>[25]</sup> Ingestion of *L. rhamnosus* (JB-1) regulated stress-induced behavior and altered GABA mRNA expression in mice.<sup>[16,17]</sup> GABA-A $\alpha$ 2 mRNA expression was reduced in the prefrontal cortex and amygdala, but GABA-A $\alpha$ 2 expression was increased in the hippocampus. Another finding was that GABA-B1b mRNA was increased in cingulate and pre-limbic cortices with concomitant reductions in expression in the hippocampus, amygdala, and locus coeruleus. In our study, we used *B. longum* and *L. rhamnosus*, as well as other probiotic strains in order to increase possibility of alterations in biochemical composition of brain of GAERS.

Earlier experimental studies on GAERS have sought to explain the underlying electrophysiological mechanism of SWDs. In one of the early reports, immunocytochemical studies demonstrated decreased beta 2 and beta 3 subunits of GABA-A receptors in the sensorimotor cortex and anterior thalamic areas in GAERS.<sup>[7]</sup> Later research also demonstrated altered GABA-A receptor activity in the nucleus reticularis thalami in GAERS.<sup>[8]</sup> There are also other studies of the role of GABA-B receptor on SWD. Increased number of GABA-B receptors was observed in the cortex of lethargic (lh/lh) mice.<sup>[8-10]</sup> Later, Princiville et al. reported up-regulation of GABA-B receptor subunits in the corticothalamic circuit in GAERS.<sup>[12]</sup>

Although GABA receptor alterations in brains of GAERS have been demonstrated, present study revealed no effect on

absence seizures in terms of cumulative number or cumulative duration in GAERS after 1-month period of probiotic consumption. However, when these results are evaluated, it is not possible to conclude that probiotics had no effect on neurotransmitter or receptor expression levels. Immunohistochemical examination of neurotransmitters and receptors was not performed in this study; therefore, possible changes cannot be excluded. The reason cumulative number and duration of absence seizures were not affected may be that the areas affected by probiotics are different from the areas that are thought to be involved in production of absence seizures. Probiotics were favored by the animals; however, this may be due to fact that the ingredients of the sachet contained other ingredients in addition to probiotics, including several vitamins.

While probiotics have beneficial effects on other diseases,<sup>[13–17,19–23,26]</sup> we found no effect on absence seizures in GAERS as result of probiotic consumption. However, it was determined that there is not enough information about the possible effects of probiotics on epileptic seizures in the literature and there is a need to conduct further studies on this topic. Such research would allow physicians to inform their patients with epilepsy how their diet and ingredients of their diet may influence their disease status. Therefore, this study could be considered a pioneer study that questions the interaction between probiotics and epilepsy and we hope that this connection can be investigated more robustly in the future.

### Limitations

Our study has some limitations. First, the number of rats used was small. However, EEG recordings were made over 3 days (total of 9 hours) for each animal, which increased the possibility to clarify difference between the 2 groups of animals.

Second, there is scant literature available for discussion and comparison of our results. A minor limitation of this study was that due to limited funding, we were unable to show the colonization of probiotic strains in the gastrointestinal tract. However, duration of animal exposure to probiotics was longer than minimum length of intake time used by several other studies.<sup>[24,27]</sup> Another important point is that our negative results might be specific to bacterial strains used in this study. Other probiotic strains of bacteria or probiotic yeasts should be investigated in further studies.

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### Disclosure

None of the authors has any conflict of interest to disclose.

### Authorship contributions

Concept: S.A., H.D.; Design: F.O., S.A., H.D.; Data collection &/ or processing: T.K., S.A., M.C.D., D. E.; Analysis and/or interpretation: F.O., T.K., S.A.; Literature search: H.D., M.C.D., D.E., S.A.; Writing: H.D., S.A., M.C.D., D.E.; Critical review: F.O.

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